OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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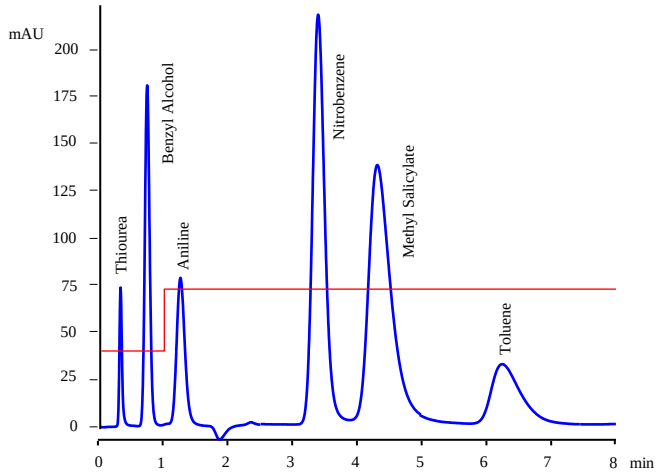
APPLICATION NOTE

STYROS™ 3R Simulated Monolith™ Polymeric Reversed Phase: Loadability Study, Comparison with Silica Reversed Phases

Reversed phase chromatography with silica provides high resolving power due to the low capacity of the stationary phase however the stability of silica is limited in aqueous buffers even at neutral pH.
The following chromatogram shows the separation of 6 small molecules on a **STYROS™ 3R Reversed Phase Simulated Monolith™** column at 30˚ C.



Chromatogram 1
Separation of 6 small molecules on
STYROS™ 3R Reversed Phase.

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ 3R** 4.6 X 50 mm
Mobile phase.	A: DI H2O (No THF) B: ACN (No THF)
Flow rate	2 ml/min (720 cm/hr. of linear flow rate)
Step Gradient	25% B for 1 min, to 40 % B in 1.1 min
Temperature	30°C
Detection	254 nm
Injection volume	6 µl
Sample:	1-Thiourea, 2- Benzyl Alcohol, 3- Aniline, 4-Nitrobenzene, 5-Methyl Salicylate, 6- Toluene (30 µg to 3.3 mg/ml each) in ACN: H2O 50:50.

The next chromatogram depicts the Loadability study of the same column using 1 to 6 µl injections.

Chromatogram 2 (next chromatogram)
Loading study on
STYROS™ 3R Reversed Phase

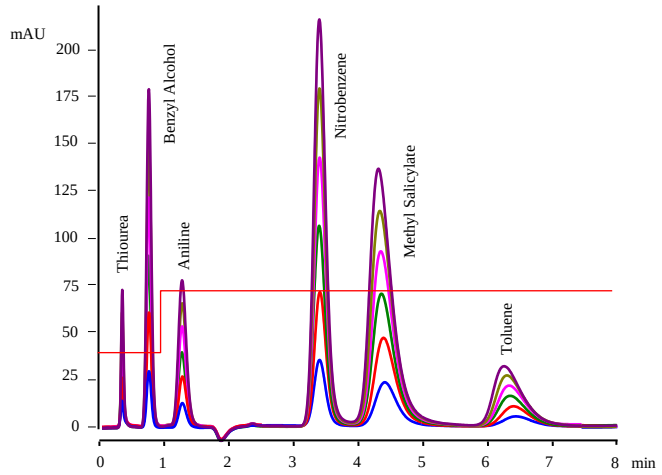
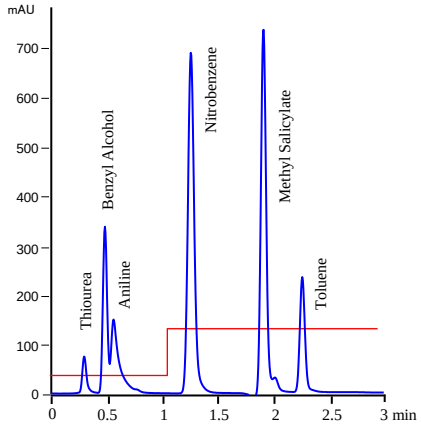


Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ 3R** 4.6 X 50 mm
Mobile phase.	A: DI H2O (No THF) B: ACN (No THF)
Flow rate	2 ml/min (720 cm/hr of linear flow rate)
Step Gradient	25% B for 1 min, to 40 % B in 1.1 min
Temperature	30°C
Detection	254 nm
Injection volumes	1 to 6 µl
Sample:	1-Thiourea, 2- Benzyl Alcohol, 3- Aniline, 4-Nitrobenzene, 5-Methyl Salicylate, 6- Toluene (30 µg to 3.3 mg/ml each) in ACN:H2O 50:50.

Under similar conditions, C18 Silica stationary phases with a similar format fail to separate all the components of the mixture. The full elution occurs under 3 minutes.



Chromatogram 3
Commerecial C18 Silica Reversed Phase
Flow Rate, 2 ml/min